

WHAT IS CLAIMED IS

546 Q1

1. A method of producing a human antibody display library, comprising:
  - providing a nonhuman transgenic animal whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produce a plurality of human antibodies;
  - isolating a population of nucleic acids encoding human antibody chains from lymphatic cells of the nonhuman transgenic animal;
  - forming a library of display packages displaying the antibody chains,
- 10 wherein a library member comprises a nucleic acid encoding an antibody chain, and the antibody chain is displayed from the package.
2. The method of claim 1, further comprising producing RNA transcripts of the nucleic acids, and translating the transcripts to form antibody chains
- 15 under conditions in which an antibody chain remains linked to the RNA transcript from which the antibody chain was translated, the complex formed between the transcript and the antibody chain constituting a library member.
3. The method of claim 1, further comprising cloning the
- 20 population of nucleic acids into multiple copies of a phage display vector and expressing the vector in host cells to form the library of display packages.
- 546 Q2
4. The method of claim 2, wherein the phage display vector is a phagemid vector.
- 25
5. The method of claim 1, wherein the nucleic acids encode variable regions of the antibody chains and the display vector comprises a segment encoding a human constant region and the cloning joins a nucleic acid encoding a variable region in-frame with the segment encoding the human constant region.
- 30
6. The method of claim 5, wherein the antibody chain is a heavy chain and the constant region comprises a C<sub>H</sub>1 region.

7. The method of claim 5, wherein the antibody chain is a light chain and the constant region comprises a  $C_k$  or  $C_\lambda$  constant region.

8. The method of claim 1, wherein the antibody chain comprises a heavy or light chain which in at least some library members is complexed to a binding partner, comprising respectively a partner light or heavy human chain to form a Fab fragment.

sub 23 9. The method of claim 1, further comprising contacting libraries members with a target, whereby library members displaying an antibody chain and binding partner (if present) with specific affinity for the target bind to the target, and separating antibody chains bound to the target to produce a subpopulation of display packages.

10. The method of claim 9, further comprising immunizing the nonhuman transgenic animal with an antigen.

11. The method of claim 10, wherein the antigen is the target or an immunogenic fragment thereof.

12. The method of claim 1, wherein a library member further comprises a nucleic acid segment encoding a tag linked to the nucleic acid encoding the antibody chain, wherein the tag is the same in different library members.

13. The method of claim 12, further comprising contacting library members with a receptor having specific affinity for the tag and isolating a subpopulation of library members that bind to immobilized receptor.

14. The method of claim 13, further comprising contacting the subpopulation of library members with a target lacking specific affinity for the tag, and isolating a further subpopulation of library members that binds to the target.

sub 24

15. The method of claim 14, further comprising subcloning ~~en~~ masses nucleic acids encoding antibody chains from the further subpopulation of library members into ~~multiple copies of an expression vector to form modified expression vectors.~~

5

16. The method of claim 15, further comprising expressing the modified expression vectors in host cells to produce a library of human antibody chains.

sub 25

10 17. A method of producing a human Fab phage display library, comprising:

providing a nonhuman transgenic animal whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produced a plurality of human antibodies;

15 isolating populations of nucleic acids respectively encoding human antibody heavy chains and human antibody light chains from lymphatic cells of the nonhuman transgenic animal;

cloning the populations into multiple copies of a phage display vector to produce a display library, wherein a library member comprises a phage capable of  
20 displaying from its outersurface a fusion protein comprising a phage coat protein, a human antibody light chain or human antibody heavy chain, wherein in at least some members, the human antibody heavy or light chain is complexed with a partner human antibody heavy or light chain, , the complex forming a Fab fragment to be screened.

25

18. The method of claim 17, wherein the plurality of human genes is free of human lambda light chain genes.

19. The method of claim 17, wherein there are no more than 40  
30 human VH genes included in the plurality of human genes.

20. The method of claim 17, wherein there are no more than 40 human VL genes included in the plurality of human genes.

21. The method of claim 17, wherein each copy of the phage display vector receives a random combination of nucleic acids encoding heavy and light chains from the respective populations.

22. The method of claim 17, wherein the populations of nucleic acids respectively encode populations of human heavy and light chain variable regions, and the phage display vector encodes human heavy and light chain constant regions expressed in frame with human heavy and light chains inserted into the vector.

10 sub a6 23. The method of claim 17, further comprising contacting libraries members from the sublibrary with a target, whereby library members displaying a Fab fragment with specific affinity for the target bind to the target, and separating phage displaying Fab fragments bound to the target to produce a further subpopulation of  
15 phage.

24. The method of claim 23, further comprising isolating a phage displaying a Fab fragment that binds to the target.

20 sub a7 25. ~~The method of claim 17, further comprising immunizing the nonhuman transgenic animal with an antigen.~~

26. The method of claim 24, further comprising expressing a Fab fragment from a phage bound to the target in soluble form.

25 27. The method of claim 17, wherein the fusion protein further comprises a tag that is the same in different library members.

28. The method of claim 27, further comprising contacting library  
30 members with a receptor that specifically binds to the tag, and isolating a subpopulation of library members bound to the receptor.

29. The method of claim 28, further comprising contacting the subpopulation of library members with a target lacking specific affinity for the tag, and isolating a further subpopulation of library members bound to the target.

5 30. The method of claim 29, further comprising subcloning a mixed population of nucleic acids encoding human antibody heavy chains and human antibody light chains from the further subpopulation of library members into multiple copies of an expression vector to produce modified expression vectors.

10 31. The method of claim 30, further comprising expressing the modified expression vectors in host cells to produce a population of human antibodies.

15 32. The method of claim 31, wherein the population of human antibodies includes at least 10 different antibodies.

33. The method of claim 32, wherein the population of human antibodies includes at least 100 different antibodies.

20 34. The method of claim 33, wherein the population of human antibodies includes at least 1000 different antibodies.

sub e<sup>8</sup> 35. A library of at least ten different nucleic segments encoding human antibody chains, wherein at least 50% of segments in the library encode human antibody chains showing at least  $10^8 M^{-1}$  affinity for the same target and no library member constitutes more than 50% of the library.

30 36. The library of claim 35, wherein the library comprises at least ten pairs of different nucleic acid segments, the members of a pair respectively encoding heavy and light human antibody chains, wherein at least 50% of the pairs encode heavy and light human antibody chains that form complexes showing specific affinity for the same target, and no pair of nucleic acid segments constitutes more than 50% of the library.

37. The library of ~~claim 36~~, wherein the library comprises at least 100 pairs of different nucleic acid segments.

38. The library of claim 37, wherein the library comprises at least 1000 pairs of different nucleic acid segments.

39. The library of ~~claim 36~~, wherein at least at least 50% of the pairs encode heavy and light chains that form complexes having affinity of at least  $10^9$   $M^{-1}$  for the target.

10

40. The library of claim 36, wherein at least 50% of the pairs encode heavy and light chains that form complexes having affinity of at least  $10^{10}$   $M^{-1}$  for the target.

15

41. The library of claim 36, wherein at least 90% of the pairs of different nucleic acid segments encode heavy and light chains that form complexes having at least  $10^9$   $M^{-1}$  affinity of the target.

20

sub a9 42. A library of at least ten different nucleic segments encoding human antibody chains, wherein at least 90% of segments in the library encode human antibody chains for the same target and no library member constitutes more than 50% of the library, and the library is free of segments encoding human lambda light chains.

25

43. A library of at least 1000 different nucleic segments encoding human antibody chains, wherein at least 90% of segments in the library encode human antibody chains for the same target and no library member constitutes more than 50% of the library, wherein each segment comprises subsequence(s) from a human VH and/or a human VL gene, and no more than 40 human VH genes and no more than 40 human VL genes are represented in the library.

30

44. A library of at least ten types of human antibodies, wherein at least 50% of the types of human antibodies in the library have an affinity of at least

$10^{10} \text{ M}^{-1}$  for the same target and no type of library member constitutes more than 25% of the library.

45. The library of claim 44 ~~having~~ <sup>comprising</sup> at least 100 different types of  
5 human antibody.

add a<sup>10</sup>